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学位（専攻分野）	博士（理学）
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学位論文題目	Studies on characteristic genome structures and on new genes found in the human MHC class Ⅲ region near the junction with the class Ⅱ
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論文内容の要旨

Genomes of higher vertebrates are composed of long-range mosaic structures of GC%, which are related to chromosome bands. Several groups, including ours, showed chromosomal G bands to be mainly composed of AT-rich sequences and T bands (an evidently heat-stable subgroup of R bands), of GC-rich sequences: ordinary R bands are heterogeneous and appear to be intermediate. Gene density, DNA replication timing, repeat sequence density and other chromosome behaviors such as recombination are related to chromosomal bands and to the long-range GC% mosaic structures.

Human MHC spans about a 4 megabase (Mb) segment of the short arm of chromosome 6 (6p21.3), and the region is composed of classes I (about 2 Mb), III (1 Mb) and II (1 Mb) from telomere to centromere. Genes in classes I and II encode polymorphic antigens involved in genetic control of immune response: Class III, which is one of the regions most densely packed with genes in the human genome, encode proteins of diverse functions mostly unrelated to immune response. Susceptibility to a large number of diseases, including autoimmune disorders, is thought related to genes in the MHC. However, in many cases, it is not clear whether the susceptibility is due to known genes or to those not yet identified.

Previous studies of our group showed the human MHC to be a long-range mosaic of GC%. Contiguous classes I and III correspond to an evidently GC-rich domain and class II, to a domain with reduced GC%. Thus, borders of Mb-level GC% mosaic domains had been assigned within under-characterized 450 kb harboring the junction of classes II and III. To precisely locate the domain border and find new genes, chromosome walking to completely cover this 450-kb area were carried out by isolating cosmid, λ phage and YAC contigs. During characterization of the 450 kb, especially of the region near the border of the Mb-level GC% mosaic domains, three human MHC class III genes were found; the gene for receptor of advanced glycosylation end products of proteins (RAGE, a member of immunoglobulin superfamily molecules believed to be related to diabetes complication), PBX2 homeobox gene (designated HOX12 by me and suspected to be a proto-oncogene), and human counterpart of mouse mammary tumor gene *int-3*, designated as NOTCH3. Human RAGE and PBX2 sequences were previously determined sequencing their cDNA clones, but the gene structures and map locations had not been known. The contiguous RAGE and PBX2 (HOX12) genes were completely sequenced in this work, and a single copy number of these genes in the human genome was shown by Southern blot analysis.

Integration of the mouse mammary tumor virus (MMTV) into the *int-3* locus promotes the transcription of flanking mouse cellular *int-3* sequence that shares significant homology with the intracellular domain of *Drosophila* neurogenic Notch gene. The human sequences found in the present work contained not only the intracellular domain part present in the *int-3* sequence but also the extracellular part present in typical Notch-family genes, showing the sequence found in this study to correspond to the human counterpart of an uninterrupted form of the transmembrane protein gene predicted for the mouse *int-3* locus. The placental cDNA

clones of the human NOTCH3 were isolated and sequenced. By constructing phylogenetic tree based on their sequences, four subfamilies for mammalian Notch genes were found.

Near a GC% transition of the long-range mosaic structures, being centromeric of NOTCH3, there were a 20 kb of dense *Alu* cluster and a 30 kb of dense LINE-1 cluster, as well as pseudoautosomal boundary-like sequence (PABL) found by Fukagawa in our group. Summary of the organization of the walked 450 kb is as follows; [Class II; AT-rich side] HLA-DRA - 140 kb - PABL - 30 kb of LINE-1 cluster - 20 kb of *Alu* cluster - NOTCH3 - PBX2 - RAGE - 90 kb - TNX (the tenascin X gene) - 70 kb - CYP21 [Class III; GC-rich side].

I have also found the gross similarity of genes on 6p21.3 and those on 9q33-q34. The human gene most closely related to NOTCH3 is TAN1 being precisely mapped on 9q34.3, that to PBX2 is PBX3 roughly mapped on 9q33-34, and that to TNX is HXB (the tenascin C gene) on 9q32-q34. By searching human Genome Data Base (GDB), not only the three genes discovered by our group but also several others on 6p21.3 were found to have counterparts mostly mapped on 9q33-q34. This gross similarity should have been brought on by duplication of a wide range of the genome and thus gives a realistic knowledge concerning evolutionary processes to built up the present human genome. The similarity is also useful for finding undiscovered genes, especially candidate genes responsible for genetic diseases.

論文の審査結果の要旨

菅谷公彦君の博士論文は、ヒトＭＨＣクラスⅡとⅢ境界のクラスⅢ側に見いだした特徴的なゲノム構造と新たに見いだされた遺伝子に関する研究についてである。ヒトなどの染色体は、バンド構造やＧＣ／ＡＴ量の異なる領域として認識されるモザイク構造を持っていると考えることができる。このようなモザイク構造は、生物進化の過程におけるゲノムと染色体の変化と系譜などの面から興味ある現象である。詳細に解析されてきたゲノム領域としてＭＨＣのクラスⅠ、ⅱ、およびⅢ領域が知られている。クラスⅡ領域はＧＣ量が低く、クラスⅢ領域は高い。したがって、この境界のゲノム構造を明らかにすることの意義は大きい。この境界に向かってクラスⅢ側からの詳細な解析は進化遺伝研究部門としてすでに開始していたが、菅谷くんはその解析を引き継いでさらに境界に近いゲノム構造を明らかにしようとした。

ゲノムのモザイク構造に関わる可能性があるものとして、菅谷君が境界近傍に発見した特有な構造は、Ａｌｕ反復配列が密に存在する領域、及びＬＩＮＥ－１反復配列の密集領域であった。このような反復配列構造が染色体とゲノムのモザイク構造の成立に関わっていることを示唆するもので、今後他の境界領域が解析されれば重要な発見に繋がる可能性が大きい。

このゲノム領域の詳細な解析の他の成果として、菅谷君はＲＡＧＥ、ＨＯＸ１２、およびＮＯＴＣＨ３の遺伝子の存在を発見し、その各々についての解析を行なった。これらはいずれも興味ある遺伝子であるが、特にＮｏｔｃｈ遺伝子群はショウジョウバエで神経細胞の分化決定に関与する遺伝子として発見され、脊椎動物では複数個のファミリーとして存在しており、それらの機能や生物進化における系譜などの観点から注目を浴びている遺伝子である。菅谷君によって、今回新たに解析されたＮＯＴＣＨ３遺伝子の構造と他のＮｏｔｃｈファミリー遺伝子との類縁関係の推定によって、この興味深い発生遺伝子群に関して重要な新しい知見を提供することになった。また、これら３遺伝子がＭＨＣ領域に配置していることを発見したことによって、この染色体部分６ｐ２１．３が、もうひとつの染色体部分９ｑ３３－ｑ３４と両方の領域に存在する８個の遺伝子にわたって密接な対応関係を持つことを明らかにした。このことは、これらの染色体とゲノム領域の生物進化における由来と系譜について、新たな推論を行なうための基礎を提供することになり、その意義は大きい。

結論として、菅谷君の博士論文はゲノム構造の進化的考察などの観点から新しい重要な知見を提供するものとなっており、十分に博士論文の要件を充たしていると判断された。